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Soil carbon sequestration and stocks: short-term impact of maize succession to cover crops in Southern Brazil Inceptisol

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Abstract

Aim of study: To evaluate soil organic carbon (SOC) sequestration and stock over the succession of maize to winter cover crops under a short-term no-tillage system.

Area of study: A subtropical area in Southern Brazil.

Material and methods: The experiment was implemented in 2013. The treatments were: seven winter cover crops single cultivated (white-oats, black-oats, annual-ryegrass, canola, vetch, fodder-radish and red-clover); an intercropping (black-oats + vetch); and a fallow, with maize in succession. Soil samples were collected after four years of experimentation, up to 0.60 m depth, for SOC determination.

Main results: SOC stocks at 0-0.6 m depth ranged from 96.2 to 107.8 t/ha. The SOC stocks (0-0.60 m depth) were higher under vetch and black-oats, with an expressive increase of 23 and 20% for C stocks in the 0.45-0.60 m layer, compared to fallow. Thus, SOC sequestration rates (0-0.60 m depth), with vetch and black oats, were 1.68 and 0.93 t/ha·yr, respectively.

Research highlights: The establishment of a high-quality and high C input cover crops in the winter, as vetch or black-oats in succession to maize, are able to increase SOC stocks, even in the short term.

Additional key words: organic matter; legumes; no-tillage; conservation agriculture; green manure; straw production; nutrient cycling.

Abbreviations used: DM (dry matter); NPK (nitrogen, phosphorus and potassium fertilization); SOC (soil organic carbon); SOM (soil organic matter); .

Authors' contributions: Conceived and designed the experiments: JTP. Performed the experiments: EB, RHR, JLL, FB and MRB. Analyzed the data: JLL, FB and JTP. Contributed reagents/materials/analysis tools: JTP, FB and RHR. Wrote the paper: JLL, FB, RHR, MRB and JTP. All author read and approved the final manuscript

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Introduction

Soil organic matter (SOM) is responsible for ensuring soil organic carbon (SOC) accumulation and plays an essential role in soil physical, chemical and biological characteristics (Zanatta *et al.*, 2019). SOC is used as an indicator of soil quality and fertility, where its concentration or stock reveals information related to how the management is affecting the system (Gregorich *et al.*, 1994; Conceição *et al.*, 2013).

Changes in SOC stocks are result from the balance between C input, mainly from plant residue or animal manure, and C output, which occurs through decomposition

(CO₂ emissions), leaching and erosion processes (Poeplau & Don, 2015). The widespread adoption of the no-tillage system contributes to the reduction of SOC losses and increases in SOC (Sá *et al.*, 2017). However, only the use of a no-tillage system does not guarantee the soil's full potential in sequestering C, requiring diverse and efficient crop systems with high residues production (Conceição *et al.*, 2013).

In this sense, the use of cover crops instead of fallow periods is an essential tool that increases the potential of the no-tillage system as a conservationist practice in sequestering SOC (Poeplau & Don, 2015; Veloso *et al.*, 2018). This is due to the large amount of SOC input via plant roots, which contributes more effectively than the

aboveground residue to SOM pool (Rasse *et al.*, 2005; Kätterer *et al.*, 2011). Also, root system diversification of cover crops contributes to nutrient cycling and reduces compaction effects (Tiecher *et al.*, 2017). Results from a meta-analysis (Poeplau & Don, 2015) shows that cover crops in agricultural areas promoted SOC sequestration of 0.32 ± 0.08 t/ha·yr.

In addition, the chemical characteristics of the residues added by cover crops are extremely important in SOC dynamics (Carvalho *et al.*, 2008). For example, low lignin content and high nitrogen concentration in plant residue, enhance decomposition processes by soil microorganisms, affecting C and nutrients release (Cotrufo *et al.*, 2013) and consequently, the development of plants grown in succession (Tiecher *et al.*, 2017). A greater diversity of crops used in rotation systems increased the quality of residue added and thus, increased SOC of a Ferralsol in humid subtropical climate (Cfa) (Jantalia *et al.*, 2003).

Moreover, these factors are dependent on soil management, as the conventional tillage enhance residue decomposition, but also increase SOM loss in comparison to no-tillage (Conceição *et al.*, 2013). In addition, climatic conditions are a key factor in these processes; typically, in subtropical environments, the residue rates of decomposition and mineralization are lower than in tropical environments (Conant *et al.*, 2011). Nevertheless, even in subtropical climate (Cfb) the inclusion of high input/quality of cover crops residues, especially legumes, in the no-tillage rotation system, can led to elevated SOC sequestration rates, as 0.50 t/ha·yr (Albuquerque *et al.*, 2015) and 0.80 t/ha·yr of C (Sá *et al.*, 2001).

Maize cultivation represents a significant area in the Brazilian subtropical region and is characterized by the high addition of residues with low nitrogen content to soil (Albuquerque *et al.*, 2015). Thus, the use of winter cover crops, grown in maize off-season can increase the soil potential of SOC sequestration, mainly by the introduction of legumes with high nitrogen addition, favoring the decomposition of maize straw (Veloso *et al.*, 2018). We hypothesized that the continuous use of maize grown in succession to winter cover crops that produce a high amount of dry mass with high quality (*i.e.* low C/N ratio) are alternatives to improve C inputs and increase SOC in a no-tillage system without the use of mineral N fertilizer. Therefore, we aimed to evaluate the C stocks and sequestration rate under the succession of maize and winter cover crops in a Haplic Inceptisol, under a no-tillage system.

Material and methods

The experiment was established in 2013, under field conditions, at the Agricultural Experimental Farm of the Universidade Federal de Santa Catarina, Curitiba, Santa Catarina state, Brazil, located at a latitude 27° 16'

24.12" S and a longitude of 50° 30' 11.80" W, with an altitude of 1050 meters. The soil in the experimental farm was classified as Haplic Inceptisol (Soil Survey Staff, 2014), clayey texture (550 g/kg of clay). When this study started, the top 20 cm soil had 28.90 g/dm³ of organic matter, a pH (CaCl₂) of 6.3, 0.0 cmol_c/kg aluminum (1 mol/L KCl), 8.35 cmol_c/kg calcium (1 mol/L KCl), 4.11 cmol_c/kg magnesium (1 mol/L KCl), 0.10 cmol_c/kg potassium (Mehlich-1) and 10.7 mg/kg phosphorus (Mehlich-1). According to Köppen-Geiger, the climate is classified as Cfb, with an average temperature of 11.6 °C in the coldest month (July) and 20.8 °C in the warmest (January), and average annual precipitation of 1500 mm.

Prior to installation of the experiment, the area was used in a conventional management system, and between 2009 and 2011 was cultivated with ryegrass (*Lolium multiflorum*) in winter, for grazing, and fallow in summer. From 2012, black oats (*Avena strigosa*) were cultivated in winter and maize in summer. The study was performed under rainfed conditions, and the experimental design was in randomized complete blocks with nine treatments and four replications. The treatments evaluated were seven cover crops single-cultivated: white oats (*Avena sativa*), black oats, ryegrass, canola (*Brassica napus*), vetch (*Vicia sativa*), fodder radish (*Raphanus sativus*) and red clover (*Trifolium pratense*); an intercropping between black oats and vetch; and a fallow treatment. The experiment consisted of 36 experimental plots, each with the dimensions of 4 m × 4 m (16 m²).

Winter cover crops sowing occurred under no-tillage system, in early May of each year, with basal fertilization with 300 kg/ha of 00-18-18 (NPK). After 75% of plant germination in the plot, 30 kg/ha of N was applied. The sowing rate of each treatment was: 80 kg/ha white oats, 7 kg/ha canola, 50 kg/ha ryegrass, 12 kg/ha red clover, 80 kg/ha of black oats, 12 kg/ha of fodder radish, 50 kg/ha of vetch, the intercropping treatment was 60 kg/ha of black oats and 30 kg/ha of vetch. The flowering was considered as the end of cover crops cycle, when the area was desiccated using glyphosate-based (N-(phosphonomethyl) glycine) herbicide (1,920 g/ha of a.i.). The winter cover crops residues were not incorporated into the soil, once the system was performed under no-tillage.

In the summer period, every year, maize was cultivated on the plots with the residues of the cover crops, always under no-tillage system. Maize sowing occurred 20-25 days after cover crops desiccation at a seed rate of 70,000 seeds/ha, with a spacing of 0.4 m between rows. P and K fertilization were with 300 kg/ha of 00-18-18. No N was applied in topdressing, aiming to verify the effect of cover plants in promoting the availability of this element. Maize was harvested in March, within the plot area (excluding 2 border lines and 1 m line at the beginning and end of the plot). Then, the remaining of the experiment area was harvested and all plant residues were deposited

in the respective plots. Grain yield data were extrapolated to kg/ha and grain humidity corrected to 13%.

Soil sampling for C determination was performed in March 2017, four years after experiment establishment. Samples were collected in one point per plot at 0-0.05, 0.05-0.10, 0.10-0.20, 0.20-0.30, 0.30-0.45 and 0.45-0.60 m depths, during maize and cover crops off-season. In the layers above 0.30 m, soil was sampled by spatula excavation, in an area delimited by a metal frame of 0.25×0.50 m. At the same point, below 0.30 m depth, soil was sampled with a screw auger (0.21 m of diameter), according to the methodology described by Blake & Hartge (1986). Each point was parallel to maize rows, between the sowing lines.

To determine the soil bulk density, all layers were carefully sampled and weighed (the entire soil mass from the layer, delimited by the metal frame, were weighed), and a subset of approximately 800 g was used for moisture and C determination. Soil moisture was determined by oven drying approximately 80 g of soil at 105 °C until constant mass. Approximately 700 g of soil was air dried and then ground to <2 mm sieve, and roots and other plant fragments were removed. Subsequently, a subset was ground to >250 µm sieve for organic C determination, which was performed by the wet combustion method, according to Walkley & Black (1934), adapted by Tedesco *et al.* (1995).

The SOC stocks were determined based on C concentration and soil density of each layer, using the equation (1) described by Sisti *et al.* (2004), where the procedure is based on the equivalent soil mass, correcting the differentiated effect that the treatments had, in relation to reference treatment (fallow), on soil density in each sampled layer.

$$C = \sum_{i=1}^{n-1} C_i + \left[M_n - \left(\sum_{i=1}^n M_i - \sum_{i=1}^n M_{Ri} \right) \right] \cdot \left[\frac{C_n}{M_n} \right] \quad (1)$$

In Equation 1, where all variables are expressed in t/ha, $\sum_{i=1}^{n-1} C_i$ is the sum of the C stock from the first to the penultimate layer; M_n is the soil mass in the deepest layer; $\sum_{i=1}^{n-1} M_i$ is the sum of the soil mass from the first to the deepest layer; $\sum_{i=1}^n M_{Ri}$ is the sum of the soil mass from the first to the deepest layer in the reference treatment; and C_n is the stock of C in the deepest layer.

To determine the C sequestration rate, fallow was used as reference treatment, according to equation (2):

$$\text{Sequestration rate} = \frac{(SOC_{\text{treatment}}) - (SOC_{\text{fallow}})}{\text{Time}_{\text{years}}} \quad (2)$$

Sequestration rate is expressed in t/ha·yr; SOC_{fallow} is the SOC stocks in fallow treatment expressed in t/ha·yr; $SOC_{\text{treatment}}$ is SOC stocks of the treatments expressed as t/ha·yr; and $\text{Time}_{\text{years}}$ is the period since the experiment establishment (4 years).

The aboveground dry matter (DM) input from cover crops was determined at the crops flowering by clipping the plants at soil surface in an area of 0.5×0.5 m. The samples were oven-dried at a temperature of 60 °C until constant mass, the residue mass was weighed and then the results were extrapolated to t/ha of DM.

The aboveground DM (t/ha) input from maize was estimated by the grain yield (obtained in this study) and the harvest index of 0.49 (Pierri *et al.*, 2016). Thus, maize aboveground dry matter was calculated as equation (3):

$$\text{Dry mass} = \frac{\text{Grain yield}}{\text{Harvest index}} - \text{Grain} \quad (3)$$

The results were submitted to the normality test (Shapiro-Wilk) and later to the analysis of variance in order to verify significant effects of the treatments. To compare the means of the treatments, the Scott-Knott test was performed at 5% of significance, using the statistical program R, package ExpDes.pt (Ferreira *et al.*, 2013).

Results

The annual aboveground DM input by cover crops and maize was not influenced ($p < 0.05$) by treatments (Table 1). On the other hand, cumulative aboveground DM input was 40.2% higher in maize + vetch and 29.9% higher in maize + black oats system, compared to maize + fallow system, the lowest DM input observed. The other treatments presented intermediate cumulative aboveground DM input, however, there was wide instability throughout the growing seasons.

The SOC concentration ranged from 13.6 to 17.3 g/kg at the superficial layers (0-0.10 m) and from 8.5 to 11.6 g/kg at the subsurface layers (0.3-0.6 m) (Table 2). Throughout the soil profile, a decreasing gradient was observed regarding the SOC concentration. There were significant variations in SOC concentrations in depth, where red clover resulted in the lowest concentrations at 0.3-0.45 m layer; and vetch and black oats resulted in the highest SOC concentrations at 0.45-0.60 m layer.

SOC stocks at 0-0.6 m depth were influenced by the treatments, with values ranging from 96.2 to 107.8 t/ha (Table 3). The cultivation of vetch and black oats in winter in succession to maize resulted in the highest SOC stocks (0-0.60 m), 107.8 and 105.2 t/ha, respectively. These cover crops mainly influenced SOC stocks in 0.45-0.60 m depth, with SOC stocks significantly higher than fallow and the other cover crops cultivated. No differences were observed in SOC stocks up to 0.45 m depth.

SOC sequestration rate at 0-0.60 m depth was strongly increased by vetch and black oats cultivation, with 1.68 and 0.93 t/ha·yr of C, respectively (Table 3). Fodder radish, canola, white oats, ryegrass, red clover and the intercropping (a mix between black oat and vetch) were

Table 1. Annual and cumulative aboveground dry matter (DM) input (t/ha) of winter cover crops and maize, during four growing seasons in a no-tillage Haplic Inceptisol (Curitibanos, Brazil).

| Treatment | 2013/2014 | | | 2014/2015 | | | 2015/2016 | | | 2016/2017 | | | CUM** [5] |
|------------------------------|-------------------------|------------------------|------------------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|--------------|
| | CC ^{ns} [2] | M ^{ns} [3] | T ^{ns} [4] | CC ^{ns} | M ^{ns} | T ^{ns} | CC ^{ns} | M ^{ns} | T ^{ns} | CC ^{ns} | M ^{ns} | T ^{ns} | |
| Vetch | 0.86 | 8.90 | 9.70 | 1.80 | 7.20 | 9.00 | 2.70 | 11.90 | 14.70 | 4.10 | 4.90 | 9.00 | 42.39 a |
| Fodder radish | 1.00 | 7.40 | 8.40 | 2.20 | 5.00 | 7.20 | 2.50 | 11.10 | 13.60 | 2.10 | 2.60 | 4.70 | 33.87 d |
| Fallow | 1.81 | 7.00 | 8.80 | 0.60 | 5.60 | 6.20 | 1.80 | 8.10 | 9.90 | 3.20 | 2.80 | 6.00 | 30.23 e |
| Black oats | 1.11 | 8.40 | 9.60 | 2.30 | 5.40 | 7.70 | 2.50 | 10.90 | 13.40 | 6.60 | 2.00 | 8.60 | 39.26 b |
| Canola | 1.11 | 8.00 | 9.10 | 1.80 | 5.50 | 7.20 | 2.10 | 11.10 | 13.20 | 2.40 | 2.50 | 4.90 | 35.01 c |
| Intercropping ^[1] | 1.13 | 7.00 | 8.20 | 1.70 | 3.70 | 5.40 | 3.10 | 8.10 | 11.20 | 3.80 | 4.90 | 8.60 | 35.33 c |
| White oats | 1.07 | 7.30 | 8.40 | 1.70 | 4.40 | 6.10 | 2.50 | 9.00 | 11.50 | 8.00 | 2.30 | 10.30 | 36.25 c |
| Ryegrass | 1.11 | 7.80 | 8.90 | 2.70 | 4.50 | 7.20 | 2.40 | 7.10 | 9.50 | 5.10 | 2.30 | 7.40 | 32.99 d |
| Red clover | 1.26 | 7.10 | 8.40 | 1.70 | 6.00 | 7.60 | 2.30 | 11.10 | 13.40 | 1.50 | 2.70 | 4.20 | 33.64 d |

^[1] Intercropping between black oat and vetch. ^[2] CC: cover crops. ^[3] M: maize. ^[4] T: total. ^[5] CUM: cumulative DM input was obtained by the sum of the four growing seasons. Different letters on the columns indicate difference by Scott-Knott test at 5% significance. ns: not significant. ** Significant at 1% probability.

not efficient, in relation to fallow, in sequestering SOC, moreover, they led to SOC depletion in an average rate of 0.76 t/ha·yr of C. As observed in SOC stocks, this difference was due to the SOC sequestration in depth, and not in the superficial soil layer (0-0.2 m).

Discussion

The cultivation of vetch and black oats as cover crops in winter, in succession to maize, increased 5.8 and 3.1% the SOC stock (0-0.6 m depth), with an expressive increase of 22.8-19.7% at 0.45-0.6 m layer when compared to fallow (Table 3). These results are interesting because even in the short term of experimentation (4 years), it is

already possible to observe positive effect of these cover crops in SOC accumulation.

This was possible due to constant annual DM input, via plant residues from these crops (Table 1), which were above the minimum required to maintain SOC stocks; which in Brazilian subtropical clayey soils under no-tillage, must be above 6.83 t/ha·yr of DM (Ferreira *et al.*, 2012). Long-term studies in southern Brazil on very clayey (Calegari *et al.*, 2008) and clayey (Albuquerque *et al.*, 2015) soils also showed the greatest effect in vetch and black oats on soil C accumulation. This indicates that the soil of the present study still has the capacity to increase SOC stocks over time. Also, it is important to highlight the high DM input from maize, which impacts in residue quality (high C/N ratio), having a direct effect on

Table 2. Soil organic carbon (SOC) concentration (g/kg) and soil bulk density (g/cm³) up to 0.6 m depth Haplic Inceptisol cultivated with winter cover crops and maize, during four growing seasons in no-tillage (Curitibanos, Brazil).

| Depth (m) | Fallow | F. rad [1] | Vetch | B. oats [2] | Canola | Inter. [3] | W. oats [4] | Ryegrass | R. clover [5] | CV [6] | BD [7] |
|-------------------------|---------|---------------|---------|----------------|---------|---------------|----------------|----------|------------------|-----------|--------------------|
| 0-0.05 ^{ns} | 16.88 | 17.04 | 17.29 | 15.55 | 16.56 | 16.93 | 15.73 | 16.29 | 15.75 | 7.33 | 1.08 ^{ns} |
| 0.05-0.10 ^{ns} | 15.38 | 13.59 | 15.07 | 13.86 | 13.82 | 14.78 | 14.71 | 14.29 | 14.05 | 7.98 | 1.41 ^{ns} |
| 0.10-0.20 ^{ns} | 14.63 | 12.75 | 13.93 | 12.95 | 13.85 | 13.89 | 12.7 | 13.81 | 13.84 | 6.25 | 1.47 ^{ns} |
| 0.20-0.30 ^{ns} | 12.9 | 11.92 | 11.57 | 12.76 | 12.15 | 12.78 | 12.33 | 12.27 | 13.4 | 8.63 | 1.45 ^{ns} |
| 0.30-0.45* | 11.18 a | 10.91 a | 11.29 a | 11.28 a | 11.68 a | 10.66 a | 11.73 a | 11.15 a | 10.3 b | 5.41 | 1.34 ^{ns} |
| 0.45-0.60** | 10.22 b | 10.9 b | 11.59 a | 10.94 a | 10.19 b | 9.02 b | 9.91 b | 10.10 b | 8.53 b | 8.86 | 1.39 ^{ns} |

^[1] Fodder radish. ^[2] Black oats. ^[3] Intercropping between black oat and vetch. ^[4] White oats. ^[5] Red clover. ^[6] Coefficient of variation (%). ^[7] Bulk density (g/cm³). ns: not significant. * Significant at 5% probability. ** Significant at 1% probability. Different letters on a row indicate difference by Scott-Knott test at 5% significance.

Table 3. Organic carbon stock (t/ha) up to 0.6 m deep and soil organic carbon (SOC) sequestration (t/ha·yr) in a Haplic Inceptisol cultivated for four years with winter cover crops, fallow and mix in succession to corn in no-tillage system (Curitibaños, Brazil).

| Depth (m) | Fallow | F. rad [2] | Vetch | B. oats [3] | Canola | Inter. [4] | W. oats [5] | Ryegrass | R. clover [6] | CV [7] |
|--|----------|---------------|----------|----------------|---------|---------------|----------------|----------|------------------|-----------|
| 0–0.05 ^{ns} | 8.50 | 8.86 | 9.50 | 8.10 | 8.63 | 8.82 | 8.19 | 8.48 | 8.21 | 7.04 |
| 0.05–0.10 ^{ns} | 10.71 | 9.88 | 11.36 | 9.98 | 9.87 | 10.61 | 10.56 | 10.17 | 10.50 | 7.81 |
| 0.10–0.20 ^{ns} | 21.22 | 20.80 | 21.15 | 19.12 | 19.52 | 20.40 | 19.24 | 21.06 | 20.35 | 5.48 |
| 0.20–0.30 ^{ns} | 18.03 | 17.45 | 17.93 | 18.83 | 18.73 | 19.19 | 18.08 | 18.30 | 19.68 | 6.60 |
| 0.30–0.45 ^{ns} | 21.13 | 22.06 | 23.76 | 22.74 | 23.47 | 22.91 | 23.79 | 22.34 | 19.79 | 7.70 |
| 0.45–0.60 ^{**} | 20.50 b | 21.14 b | 24.63 a | 24.00 a | 20.74 b | 19.57 b | 20.67 b | 20.78 b | 19.15 b | 9.45 |
| 0–0.20 ^{ns} | 41.36 | 37.68 | 41.48 | 37.21 | 38.92 | 39.83 | 37.40 | 39.10 | 38.62 | 5.41 |
| 0–0.60 [*] | 101.90 b | 101.79 b | 107.80 a | 105.16 a | 97.33 b | 101.5 b | 99.94 b | 100.14 b | 96.17 b | 4.25 |
| C sequestration (0–0.2) [1] ^{ns} | - | -1.05 | 0.03 | -1.18 | -0.69 | -0.43 | -1.13 | -0.65 | -0.78 | - |
| C sequestration (0–0.6) ^{**} | - | -0.03 b | 1.68 a | 0.93 a | -1.30 b | -0.56 b | -0.56 b | -0.50 b | -1.63 b | - |

[1] SOC sequestration was calculated by the difference between SOC stocks of the treatment and SOC stocks in fallow and divided by experiment duration (four years). [2] Fodder radish. [3] Black oats. [4] Intercropping between black oat and vetch. [5] White oats. [6] Red clover. [7] Coefficient of variation (%). Different letters on a row indicates difference by Scott-Knott test at 5% significance. ns: not significant. * Significant at 5% probability. ** Significant at 1% probability.

the decomposition rate and on the soil C stock, especially in the short/medium term evaluation.

Long-term experiments (17 years) in the state of Paraná, Brazil (Santos *et al.*, 2011), showed higher SOC concentrations than in the present study, but with similar patterns. According to the authors, the lowest SOC concentration (24 g/kg) in the superficial layers occurred in the soybean-wheat succession, characterized by a low amount of residue addition. However, a biennial rotation between ryegrass-maize and ryegrass-soybean promoted the largest increases in SOC (30 g/kg) (Santos *et al.*, 2011), which is related to higher residues addition by these crops, mainly maize, as well as the high proportion of roots produced by ryegrass. Albuquerque *et al.* (2015) also found increases in SOC in depth when black oats or vetch were used as cover crops, which is related to the expressive root development of these crops, accumulating C in depth. These results contrast with those from red clover cultivation, which resulted in the lower SOC concentrations (Table 2), that are explained by its low residue input along the experiment (Table 1). In addition, this crop is characterized by lower root production in depth (Heuermann *et al.*, 2019), which may have contributed to the lower SOC concentrations at 0.45–0.60 m layer.

On the other hand, vetch cultivation also had positive effects on maize DM production (Table 2), mainly by the higher residue quality (higher N content), supplying N through straw decomposition and release to maize uptake (Veloso *et al.*, 2018). The low C/N ratio of vetch accelerates straw decomposition rate and nutrient cycling, favoring greater incorporation of C in soil (Moreira & Siquei-

ra, 2002), as well as promoting further development of the crops grown in succession. Gonçalves & Ceretta (1999) found similar responses, where the cultivation of legumes (vetch and blue lupine) in succession to maize provided the largest SOC stock after a 6-year trial period.

In our study, we observed high adaptability of black oats to the climate and soil of the experiment, reaching DM production even higher than some studies in similar conditions (Ceretta *et al.*, 2002), evidencing its rusticity and potential use in areas with little technological advance or low use of fertilizers. While fodder radish, canola and red clover had difficulty in the establishment and development of crops, reflecting in low DM from these cover crops (Table 1), thus limiting the expressiveness of the maximum production potential. In turn, Calegari *et al.* (2008) found positive effects of fodder radish to increase SOC stocks, which indicates that there may be other factors influencing the proper establishment of this crop in the studied conditions.

The high SOC sequestration rates under vetch and black oats (Table 3) are due to the expressive effect that occurred in the deeper layers (0.45–0.60 m). This reinforces our assumption that these crops ensured significant participation in C increment via root system, whereas red clover, for example, with shallow root system had the opposite effect, presenting the highest SOC depletion rate. In addition, the lack of differences in SOC sequestration rates in surface layers can be partly attributed to the high natural fertility of the soil in question, where changes in SOC are slowly expressed, without major changes over time (Hassink, 1997).

The two crops, that when single-cultivated promoted the highest SOC stocks (vetch and black oats), had

no effect on SOC when cultivated in an intercropping (Table 3). In the intercropping, both winter and summer DM input by crops were low (Table 1), with amounts similar to the fallow treatment. This condition led to a great SOC depletion. In the intercropping system, the reduction in the amount of vetch seeds in relation to its single cultivation may have reduced its potential to fix N per unit area. In the same way, the reduction of the black oats seed rate reduced the final sward density, thus reflecting in lower biomass production and input to soil (Van Kessel & Hartley, 2000). Additionally, the intercropping intensifies competition for essential resources, such as water, light and nutrients; and affects the initial establishment and final stand of the plants, which can contribute to lower yields in DM (Hauggaard-Nielsen *et al.*, 2006).

The multiple benefits of increasing SOM are well discussed in the literature. In addition to climate regulation, SOM plays a key role in soil fertility and crop yields (Tiessen *et al.*, 1994; Oldfield *et al.*, 2019), improves soil physical quality (soil aggregation and water holding capacity) (Oldfield *et al.*, 2018), contributes to ecological intensification (ecosystem services) (Garrat *et al.*, 2018), increases yield stability of crops and consequently food security (Pan *et al.*, 2009; Lal, 2010). The results observed in this study stand out by two main reasons. The first, shows that it is possible to increase SOM even in a short-term period, once the correct succession system is established. The second, that in some cases (when other factors are not limiting) we can improve it (SOM) even when external mineral fertilizers are limited.

The results show that although crop diversification is a recommended practice, it is essential to choose species adapted to the region that produce satisfactory amounts of DM, aiming at maintaining or increasing soil C levels.

The cultivation of vetch or black oats as winter cover crops in succession to maize promotes soil C sequestration, indicating that this management is efficient in comparison to fallow areas. This occurs mainly due to high annual aboveground DM input by these crops.

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